UNIVERSITY OF CALIFORNIA PUBLICATIONS

COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
BERKELEY, CALIFORNIA

BACTERIAL DECOMPOSITION OF OLIVES DURING PICKLING

BY

W. V. CRUESS AND E. H. GUTHIER

BULLETIN No. 368

JULY, 1923

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA
1923

BACTERIAL DECOMPOSITION OF OLIVES DURING PICKLING

By W. V. CRUESS and E. H. GUTHIER

Picklers of ripe olives, since the beginning of the industry in California, have suffered loss of fruit during pickling because of bacterial decomposition. The financial loss during the 1921 season from this cause was exceptionally severe, probably in excess of $75,000. The loss was not so serious during the 1922 season, largely because of the introduction of control measures by our investigations.

The investigations* reported in this bulletin were made during the pickling seasons of 1921–22 and 1922–23.

Nature of the Decomposition.—In the usual process of pickling ripe olives, the fruit is first treated with a dilute solution of lye (sodium hydroxide) long enough to permit the lye to penetrate the skin and a short distance into the flesh. The olives are then exposed to the air in the vats until the color is darkened by oxidation. The first exposure usually lasts three days or longer. The olives are then treated with several applications of dilute lye and are exposed to the air between these applications for periods of about 24 hours each. The final lye treatment is prolonged sufficiently to destroy all bitterness. Water is then placed on the fruit and is changed several times daily until the lye is completely washed from the tissues. Dilute brine is then placed on the olives for two days or longer. The fruit is then sorted, canned and sterilized at 240° F. for 40 minutes.

The decomposition occurs more frequently during the washing of the fruit following the final lye treatment than at any other stage of the pickling process.

During the first stages of the disease the fruit develops a characteristic sour odor and flavor. As the disease progresses, the color bleaches and gas is formed in the olives and in the brine. "Floaters" (fermenting olives) appear on the surface of the water or brine in the pickling vats and bleaching becomes more pronounced. Finally putrefaction occurs and the olives become soft and inedible. Gas pockets develop in the fruit and are the immediate cause of floating. The appearance of such fruit is shown in figure 1.

* Grateful acknowledgment is made to P. H. Waldruff, L. F. Lingle and to many packers and processors for their coöperation and for fruit used in the investigations.

The Organisms Responsible for the Disease.—Microscopical examination of numerous samples of fermented olives has revealed the presence of large numbers of bacteria. The predominant organism was found to be a very short rod, occurring singly or in pairs. A long rod-like bacterium was also present in appreciable numbers. See figure 2 for the microscopical appearance of these organisms.

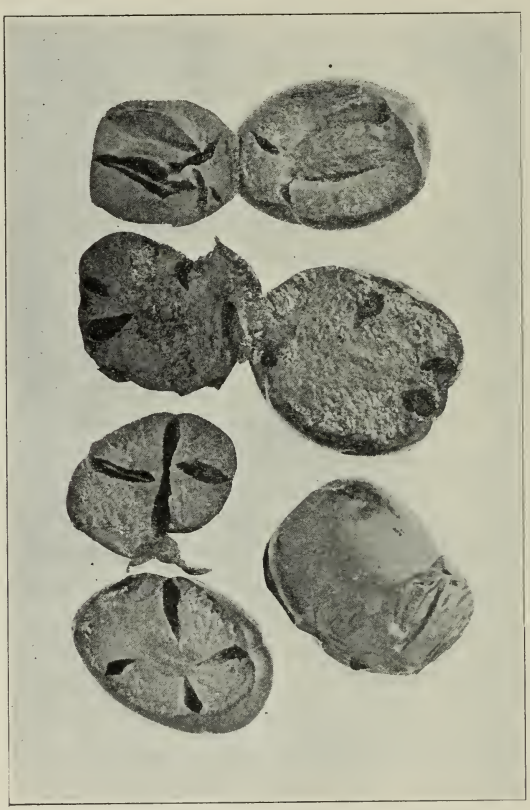

Fig. 1.—Olives showing effects of bacterial decomposition. Some of these olives are cut to show gas pockets.

It was found possible to reproduce the disease in sterilized olives by inoculation with the juice from affected olives or by addition of such olives to the unaffected fruit.

Pure cultures of the organisms from fermenting olives were prepared by P. H. Waldruff in 1922 and by Geo. Henny in 1923. More than twenty different types were obtained. Waldruff found that only two of his pure cultures were capable of causing the disease and these two only when used in combination. One of these organisms

was an anaerobe and the other an aerobe. Henny obtained several pure cultures which produced gas. The senior author during the 1921 season prepared twelve pure cultures of aerobic bacteria but of these none were capable of causing gas formation in olives, although several caused bleaching of the color and formation of acid.

It is, therefore, probable that the disease is caused by several types rather than by a single type of bacteria. Some varieties cause bleaching and produce acid but no gas; others produce both acid and gas.

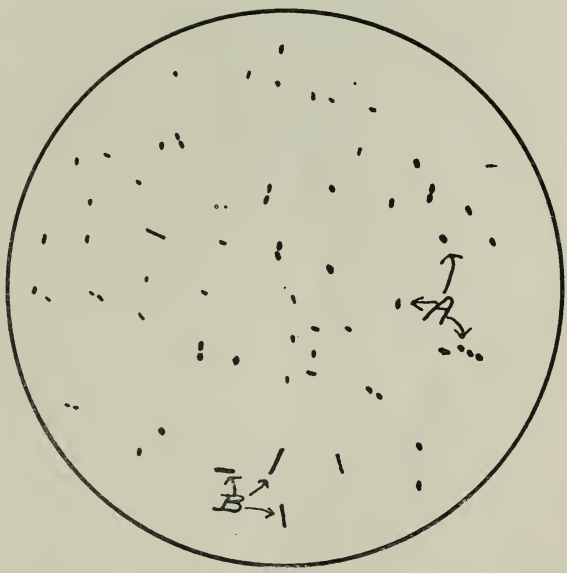

Fig. 2.—Organisms from olives undergoing decomposition during pickling. Magnified about 1000 times.

Sterilized olives were inoculated with cultures of *Bacillus subtilis, Bacillus coli* and *Bacillus vulgatus,* because it was suspected that one of these organisms might be responsible for the disease. All failed to produce gas, although growth was vigorous.

The resistance to heat of the organisms from fermenting olives and to sodium hydroxide is discussed later in this publication.

Sources of Infection.—In order to determine the possible sources of the bacteria, pickled olives were sterilized in 3 per cent brine in bottles plugged with cotton. To different samples were added: (1) water from a well at Lindsay; (2) olives from trees at Lindsay; (3) unpickled olives from holding solution (olives grown at Lindsay); (4) piece of concrete vat in which olives had spoiled during pickling;

(5) piece of stave of barrel in which unpickled olives had been shipped; and (6) piece of wooden vat in which olives had spoiled during pickling.

Sample (1) gave a negative result; samples (2), (3), (4) and (6) caused typical fermentation and spoiling when tested during the 1921 season; samples (5) and (6) were tested during the 1922 season, and, although putrefaction occurred, gas formation was absent.

Brine and water from fermenting olives were added to sound olives and typical fermentation, floating and softening ensued. Unpickled olives after one month's storage in brine caused the disease to develop when added to sterile pickled olives.

These results indicate that the bacteria are present on or in the olives before they enter the factories. It is also evident that tanks in which spoiling has occurred can infect sound olives placed in them. Olive picklers have also observed that olives soften and ferment more frequently in vats in which such spoiling has previously occurred.

Inoculation of sound vats of olives undoubtedly frequently occurs by transfer of bacteria from one vat to another on the hands of the pickler or on the equipment used for stirring the olives. Picklers, therefore, should attempt to reduce this source of contamination to a minimum by sterilizing dippers, paddles and air hose in a disinfectant solution, such as 5 per cent formalin, after contact with fermenting olives.

Relative Susceptibility of Different Varieties of Olives.—In our experiments it was observed that the Sevillano, Ascolano and Manzanillo olives are more susceptible than the Mission to bacterial spoiling during pickling; the relative order of susceptibility being Sevillano, Ascolano, Manzanillo and Mission. It was found very difficult to pickle fresh Sevillano olives in the laboratory without loss of much of the fruit by bacterial decomposition.

Little difficulty was experienced in causing fermentation and floating of Sevillano, Ascolano and Manzanillo olives experimentally; Mission olives required heavier inoculations and much longer periods of incubation to produce the disease. After the appearance of fermentation, softening progressed more rapidly in the Sevillano, Ascolano and Manzanillo varieties than in the Mission.

Effect of Temperature During Pickling.—Losses from bacterial spoiling of Manzanillo olives have been heaviest in factories in which the water used for washing is above 70° F. In factories in which the vat room and water are below 60° F. losses in most instances from this source have been small.

In order to obtain more accurate information on the relation of temperature to bacterial decomposition the four most important varieties of olives were pickled to the point of application of wash water. In one experiment, samples of Mission, Manzanillo and Ascolano olives were stored at temperatures of 60° F., 80° F., and at room

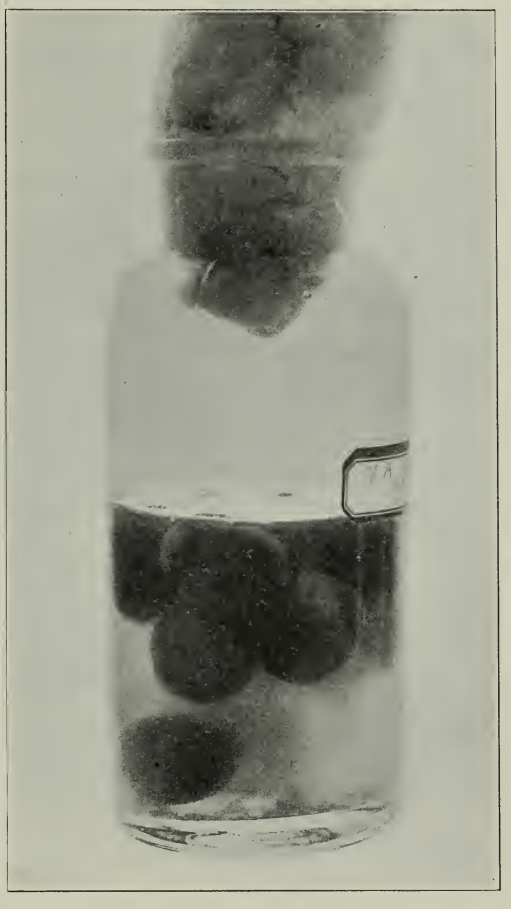

Fig. 3.—Fermenting olives showing floating of the fruit and surface growth.

temperature (50–70° F.). The olives were inoculated. Fermentation became evident at 80° F. in less than 36 hours in all three varieties; in the Ascolanos at room temperature in less than 45 hours; and in the Manzanillos at room temperature in less than 67 hours, and at 60° F. in 144 hours. The Mission olives did not develop fermentation, either at room temperature or at 60° F., during the period (6 days) required to wash the lye from the olive tissues.

In another experiment Mission and Manzanillo olives were washed at 60° F. and at 78° F., respectively. Fermentation of the Manzanillos at 78° F. became evident within 4 days, and only after 16 days at 60° F. The Missions failed to develop fermentation at 60° F. and required more than 7 days storage at 78° F. to develop fermentation.

Waldruff was unable to cause fermentation of non-inoculated Manzanillo olives at 60° F., whereas fermentation at room temperature occurred in less than one week in olives of the same lot.

Sevillano olives fermented readily at 60° F. In vats in a commercial factory, Sevillanos were observed to be fermenting at 45° F.

Other experiments confirmed our observation that Mission and Manzanillo olives are much less susceptible to bacterial spoiling at temperatures below, than at those above, 60° F.

In two factories which have experienced heavy losses from bacterial decomposition of olives during pickling, the temperature of the water used in the vat room was from 72–76° F. In three other factories in which bacterial spoiling during pickling has been rare and losses from this cause have been almost unknown, the temperature of the water in the pickling vats was less than 50° F.

It seems evident that the temperature of the wash water is a very important, if not the most important, cause for the development of bacterial decomposition in olives during pickling. Those who contemplate the establishment of olive pickling factories should determine the temperature of the water available for use in pickling. Whether artificial cooling of the water would be economical or not has not been determined in our investigations. It is possible that other means of preventing bacterial decomposition discussed later in this publication would be more feasible.

Effect of Storing Fruit in Brine Before Pickling.—It was observed that of two factories in the same city and using water of approximately the same temperature (70–75° F.), one suffered very heavy losses and the other lost relatively little fruit by bacterial decomposition. Apparently, the principal difference in the pickling processes in the two factories was in the treatment before pickling. In the factory in which losses were severe, much of the fruit was pickled direct from the tree or after a short storage in brine; in the other factory it was held in brine for several weeks to several months before pickling.

A preliminary experiment made in the 1921–22 season, indicated that Manzanillo olives pickled direct from the tree underwent fermentation and softening readily, whereas those pickled after six months' storage in brine failed to develop these symptoms, even when heavily

inoculated. During the 1922–23 season 21 separate lots of olives of the leading varieties were pickled both before and after storage in brine ("holding solution"). Fifty-four lots of olives were pickled in the 21 experiments. Brines ranging from 3 to 12 per cent salt were used. In one experiment the olives were stored in water only.

The storage period ranged from 26 to 120 days. Fermentation occurred in the unpickled fruit during storage in brine, but apparently did not injure its texture. Olives placed in 12 per cent salt solution fermented slowly for the first few days of storage, but as the brine became more dilute through osmosis, fermentation became more vigorous.

The brines in all the samples decreased in salt concentration by osmosis. For this reason after 7 to 15 days' storage, salt was added to increase the brines to the original concentrations and periodically thereafter to maintain the original concentrations.

The various samples were pickled by the usual lye and air exposure process described on page one. During washing to remove the lye the olives were inoculated with crushed fermenting olives to induce fermentation and softening in susceptible olives.

All experimental lots of olives not previously stored in brine developed the disease very quickly and in a vigorous form. Those stored 26 days or longer in brine either did not develop the disease, or developed it only after prolonged standing, e.g. 15 to 18 days. The results were very striking and conclusive. Olives of several lots which failed to ferment were sterilized in a 3 per cent salt solution containing 1 per cent of sugar. After inoculation, fermentation occurred within 3 days at room temperature, indicating that sugar favors fermentation.

Olives stored in brines of 10 to 12 per cent salt were firmer when pickled than those stored in brines of 3 to 6 per cent, or pickled without storage in brine.

It is probable that storage in brine before pickling permits decomposition of sugar and other fermentable substances so that when the fruit is pickled later there are none of these materials to support fermentation. Fermentation of the unpickled fruit apparently did not injure it.

CONTROL MEASURES

Several methods of preventing and checking the disease during the pickling process were studied, both in the laboratory and under factory conditions.

Hastening the Washing Process.—Fermentation and softening most frequently occur during the washing of the olives in water to

remove the lye. Therefore, our efforts to shorten the pickling process were applied principally to this step in the work.

Olives of the same variety were pickled to the point of adding the first wash water. They were divided into five lots and water applied at 140° F., 97° F., 77° F., 67° F., and 37° F., respectively. The water was changed twice daily on all lots except that at 140° F., on which it was changed three times daily. All lots except that at 140° F. were maintained constantly at the respective temperatures. Water at 140° F. was placed on this lot of olives, but it was allowed to cool naturally to 75–100° F. between additions of water.

The times necessary at each temperature to completely remove the lye from the flesh of the fruit are shown graphically in figure 4.

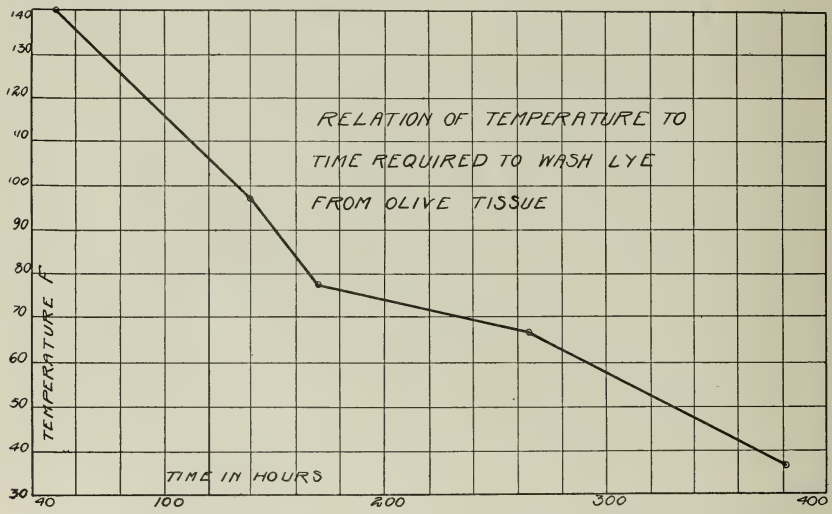

Fig. 4.—Effect of temperature of wash water on time required to remove lye from olive tissue.

At 97°, 77°, and 67° F. fermentation occurred but not at 37° F. nor at 140° F. It is probable that a temperature of 140° F. retarded bacterial growth, in addition to greatly hastening the washing process.

One factory has used wash water at about 140° F. with success.

Pasteurization.—A large number of experiments were made during the past two seasons to determine the death temperature of the organisms responsible for the disease. Two experiments will be cited to illustrate the methods used.

In Experiment 390, fermenting pickled olives were crushed in a sterile mortar and pressed through sterile cheesecloth. The juice was mixed with sterile water. Samples were heated to 175° F. for 30

and 45 minutes, respectively. Surface cultures of the heated and of the untreated samples were made on nutrient-dextrose agar agar to determine the total number of aerobic bacteria present, and deep cultures in dextrose agar agar to determine whether or not gas forming bacteria was present.

Fig. 5.—Effect of pasteurization on organisms from fermenting olives. No. 1, heated to 175° F. for 30 minutes; No. 2, not heated. Note breaking of the agar agar jelly in No. 2 by gas.

The untreated sample contained 13,000,000 aerobic organisms per c.c.; those heated to 175° F. for 30 and 45 minutes contained no aerobic bacteria.

The deep culture of the untreated sample quickly developed gas; that from the sample heated to 175° F. for 30 minutes developed a

single colony from 1 c.c. of sample but no gas, and that heated to 175° F. for 45 minutes developed neither colonies nor gas. Figure 5 illustrates the appearance of the deep cultures from an untreated check (No. 2) and from the sample heated to 175° F. for 30 minutes (No. 1). Note breaking of the agar agar in sample No. 2, positive evidence of the presence of gas producing organisms.

Fermenting pickled Manzanillo olives were divided into ten lots which were then heated to 212° F. for 15 minutes; 190° F. for 15 minutes; 170° F. for 15, 30, 45, and 60 minutes; and to 150° F. for 15, 30, 45 and 60 minutes, respectively. The samples were then stored in sterile bottles plugged with cotton for about 30 days. Samples heated to 212° F. for 15 minutes; 190° F. for 15 minutes; 170° F. for 60 minutes, and 150° F. for 60 minutes did not develop fermentation. All other samples fermented in less than seven days. Other experiments confirmed these results.

A longer period of heating was found necessary with the whole olives than with the juice, probably because considerable time was required for the flesh near the pits of the olives to reach the temperature of the surrounding heated liquid. A temperature of 190° F. for a period of 15 minutes would appear to be the most economical for pasteurizing.

In commercial factories, pasteurizing fermenting olives at 175° F. for 30 minutes was found effective in checking fermentation long enough to remove the lye from the olives by washing with water in the usual manner. The method, therefore, has proved to be applicable to factory practice.

Fermenting olives exhibited some softening when pasteurized in the vats by direct steam, because of mechanical bruising by the steam jet. Some method of heating the water or brine outside the vat should be employed.

Olives which contained large gas pockets and were floating usually softened when heated. Therefore, pasteurization should be applied before fermentation has progressed to the point of causing floating of the olives. Pasteurized olives should also be sorted in some cases to remove softened fruit. The softened fruit can usually be identified by its collapsed or wrinkled appearance after heating.

Control by Lye Treatment.—In the laboratory, fermenting pickled olives were treated for various lengths of time with lye solutions varying from ¼ per cent to 1½ per cent sodium hydroxide (lye). The ¼ per cent and ½ per cent solutions failed to prevent subsequent fermentation during washing; ¾ per cent and stronger solutions

applied for about 15 hours prevented subsequent fermentation, but application for 3 to 8 hours in most cases failed to check the disease.

Treatment of actively fermenting olives in the vats in factory scale experiments with ½ to 1 per cent lye solutions to the pits (about 3 to 5 hours application) failed to check fermentation long enough to permit washing out of the lye in the usual manner. Similar treatment of olives in the first stages of fermentation, however, has proved effective in checking the disease in many instances.

In one experiment fermenting olives were crushed and pressed with sterile equipment and the juice was mixed with sterile water. Samples of the liquid were treated for 35 minutes and for 3 hours with ¼, ½, and ¾ per cent lye solutions. Counts of surviving bacteria were made by planting on nutrient agar agar and qualitative tests for the presence of gas forming bacteria were made in dextrose agar tubes. The ¼, ½, and ¾ per cent lye solutions for 3 hours destroyed the gas forming bacteria, but a few non-gas forming organisms survived.

In another experiment fermenting olives of the same lot were treated at room temperature for 5 hours and for 23 hours with lye solutions of ½, ¾, and 1 per cent. No gas forming bacteria were recovered from any of the samples after 23 hours treatment and after 5 hours treatment with 1 per cent lye solution. The numbers of aerobic bacteria in the olives before and after treatment are shown in the following table.

TABLE 1

EFFECT OF LYE CONCENTRATION AND LENGTH OF LYE TREATMENT ON AEROBIC BACTERIA IN FERMENTING OLIVES

Sample	Aerobic bacteria per c.c.	
	After 5 hours treatment	After 23 hours treatment
Untreated	425,000,000	425,000,000*
½ per cent lye	320,000	More than 40,000
¾ per cent lye	960,000	80
1 per cent lye	0	0

* Assumed to be the same as on the previous day.

In several other experiments these results were confirmed, but in one experiment treatment of the olives with a ¾ per cent solution for 24 hours failed to prevent fermentation of the olives during subsequent washing.

It seems evident from our experiments that a ¾ per cent lye solution applied for 20 to 24 hours, or a 1 per cent lye solution applied

for a shorter time, will "cure" mild cases of the disease, but that this treatment is not adequate for severe cases.

Control by Pasteurization and Subsequent Lye Treatment.—Bacterial growth in olives during washing causes bleaching; probably by the production of acid and reduction of the color (the reverse of oxidation). It was found that most of the lost color returned when the olives were given a light lye treatment and exposed to the air for 24 hours.

Because a light lye treatment used alone failed to permanently check fermentation, it was found advisable first to pasteurize the fermenting olives, then to apply a dilute lye solution (½ per cent) to the pit (2 to 4 hours application), expose them to the air for 24 hours, and wash free from lye in the usual manner.

SUMMARY

1. Bacterial decomposition (fermentation, floating, and softening) of olives has in the past caused serious loss to olive packers.

2. It is caused by gas-forming and other bacteria which are present on the surface or in the fresh fruit, the vats and other equipment before pickling.

3. The presence of fermentable material (sugars or similar compounds) in the fruit is essential for the typical development of the disease.

4. Storage in brine for four weeks or longer permits fermentation of this material before pickling without injury to the fruit and thereby renders the fruit resistant to fermentation during pickling.

5. Sevillano, Ascolano, and Manzanillo olives are much more susceptible than the Mission to bacterial decomposition during pickling.

6. The use of wash water above 60° F. greatly increases the susceptibiiity of the olives to fermentation.

7. Pasteurization at 212° F. or 190° F. for 15 minutes or at 175–170° F. for 60 minutes checks fermentation long enough to permit washing the lye from the tissues of the olives. Pasteurization at 175° F. for 30 minutes has proved efficacious in control of the disease under factory conditions.

8. Application of ¾–1 per cent lye for 23 to 24 hours checks the disease in mild cases long enough to permit completion of the pickling process, but is not sufficient for cure of advanced cases.

9. Application of dilute lye and exposure to air after pasteurization improves the color of olives bleached by bacterial action.

10. Application of wash water at 140° F. reduces the time necessary for washing lye from the olive tissues to about two days. Six to eight days is normally required at 50–60° F.

RECOMMENDATIONS

1. Manzanillo, Sevillano and Ascolano olives should be stored in brine for at least 25 days before pickling. A brine of about 6–7 per cent salt (24–28° salometer) for the first week with an increase to 10 per cent (40° salometer) for the remainder of the storage period is recommended.

2. In establishing a new olive pickling factory, the prospective builder should make certain that water not above 60° F. is available.

3. In factories where the wash water is cold (40–50° F.) it is recommended that fermentation be arrested by lye treatment.

4. In factories using warm water (60° F. or above) olives fermenting during pickling should be pasteurized at 190° F. for 15 minutes; cooled to room temperature; treated with dilute lye (¼–½ per cent) to the pit; exposed 24 hours to the air to darken, and washed free from lye as rapidly as possible.

5. In factories in which bacterial decomposition has become thoroughly established, the vats should be sterilized with boiling water and great care exercised in order to avoid transfer of bacteria from infected to sound vats of olives by the hands, stirring paddles, or other implements.

6. In such factories the washing process should be hastened as much as possible, one effective means being the application of hot wash water (140° F.) to hasten leaching of lye from the olives. The fruit should be stored no longer than necessary in dilute brine after pickling, and should be canned as soon as possible when free from lye. Storage for two days in brine before canning should be the maximum in factories in which bacterial decomposition is a serious problem.